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SPECIES OF *MYXOBOLUS* (MYXOZOA) FROM THE BULBUS ARTERIOSUS OF CENTRARCHID FISHES IN NORTH AMERICA, WITH A DESCRIPTION OF TWO NEW SPECIES

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ABSTRACT: Three species of *Myxobolus* (Myxozoa, Myxosporea) occur in heart tissue of centrarchids. *Myxobolus paralintoni* Li and Desser, 1985 from *Lepomis gibbosus* in Algonquin Park and in Lake Erie, Ontario, has subcircular spores (in plane of spore length) in frontal view (11–13 μm long, 9–10 μm wide, and 5 μm thick) with a width-to-length ratio of 1:1.2. *Myxobolus jollimorei* n. sp. from *Lepomis macrochirus* in Lake Erie and in the Pascagoula River System, Mississippi, has subcircular spores (in plane of spore width) in frontal view (10.0–11.5 μm long, 12.0–14.5 μm wide, and 6.5–8.0 μm thick) with a width-to-length ratio of 1:0.8. *Myxobolus manueli* n. sp. from *Pomoxis nigromaculatus* in Lake Erie has spores (10–11 μm long, 8–10 μm wide, and 6.5–7.0 μm thick) that are nearly circular in frontal view but that have 2 distinct sublateral knobs along the sutural ridge and a width-to-length ratio of 1:1.2. All 3 species occur in the bulbus arteriosus of their hosts where they form small, saucer-shaped pseudocysts. Free spores were found free in the lumen of the heart and bulbus arteriosus, in bile, and in kidney tissue presses.

As a group, histozoic species of *Myxobolus* Bütschli, 1882 (Myxosporea) infect a diverse set of specific tissues that can include specifically the tegument, eyes, gills, skeleton, glands, kidneys, gonads, scale epithelium, muscle, digestive tract, and nervous system (Shulman, 1966). Although spores have been reported commonly from heart tissue, development of pseudocysts of species of *Myxobolus* in heart tissue is rare (Bauer et al., 1991; Masoumian et al., 1996).

The present study examines 3 species of *Myxobolus* from the bulbus arteriosus of centrarchid fishes in North America. *Myxobolus paralintoni* Li and Desser, 1985 is reported from *Lepomis gibbosus* 2 new species are described, 1 from *Lepomis macrochirus* and 1 from *Pomoxis nigromaculatus*.

MATERIALS AND METHODS

Juvenile and adult pumpkinseed (*L. gibbosus*) were collected 21 June 1994 from Lake Sasajewan (45°35'N; 78°30'W) by setting baited minnow traps. The 13 trapped fish were transported live to the nearby Harkness Fisheries Research Laboratory where they were necropsied the same day. Photomicrographs of fresh spores mounted in 1% agar were prepared during the necropsies. Infected hearts were fixed in 10% phosphate-buffered formalin and later embedded in Paraplast for preparation of histological sections. Sections (7 μm thick) were stained with Harris' hematoxylin and eosin or with Giemsa. Juvenile and adult bluegill (*L. macrochirus*) were collected 15 September 1994 and 19–26 February 1996 by netting with a seine and angling with hook and line in the Pascagoula River and joining bayous near Vancleave, Mississippi. The September sample of bluegill was fixed immediately in 10% phosphate-buffered formalin and necropsied later. Heart tissue was sectioned as described above. Photomicrographs were prepared of fixed spores stabilized in 1% agar mounts. The February sample of bluegill was necropsied fresh. Two specimens of black crappie (*P. nigromaculatus*) were collected 29 June 1993 by seine netting on a beach near Wheatley, Lake Erie. On 28 March 1995, *L. gibbosus* (7 specimens), *L. macrochirus* (7), and *Pomoxis macrochirus* (5) were collected by trap-netting in Inner Long Point Bay, Lake Erie. These fish were frozen and examined later. All measurements of pseudocysts and spores are presented in micrometers as a mean \pm SD followed in parentheses by the range.

DESCRIPTION

Myxobolus jollimorei n. sp.

(Figs. 1–4, 13)

Description: Pseudocyst gray, spherical to saucer-shaped, 50–300 in diameter. Plasmodium lacking typical ectoplasm near periphery, with spores loosely arranged within interior. Fixed spores ($n = 15$) subcircular (perpendicular to plane of spore length) in frontal view, 11.0 ± 0.5 (10.5–11.5) long, 13.8 ± 0.7 (12.0–14.5) wide, 7.5 ± 0.6 (6.5–8.0) thick. Spore width-to-length ratio $1:0.8 \pm 0.5$. Polar capsules broadly pyriform and equal in size, 6.0 ± 0.3 (5.5–6.0) long, 3.8 ± 0.3 (3.5–4.5) wide. Filament coils 6–9, wound tightly and perpendicular to longitudinal axis of capsule. Intercapsular appendix absent. Sutural ridge folds 6–8, distributed evenly around spore margin. Iodinophilous vacuole and mucous envelope lacking.

Taxonomic summary

Type host: Bluegill, *L. macrochirus* Rafinesque, 1819 (Centrarchidae).

Site of infection: Bulbus arteriosus, within the epipericardium and underlying smooth muscle and elastin. Free spores in kidney tissue.

Type locality: Pascagoula River and associated bayous near Vancleave, Jackson County, Mississippi; other localities: Inner Long Point Bay, Lake Erie, Ontario.

Type specimens: syntypes are spores in Giemsa stained sections in U.S. National Parasite Collection, Beltsville, Maryland, USNPC no. 87588.

Prevalence and intensity of infection: 86% (6 of 7) of the bluegill (17–18 cm in total length) collected from Inner Long Point Bay and 33% (2 of 6) of the bluegill (5.2–8.5 cm in total length) collected from the Pascagoula River system 9 September 1995. Intensity of pseudocysts ranged from 1 to 30 pseudocysts.

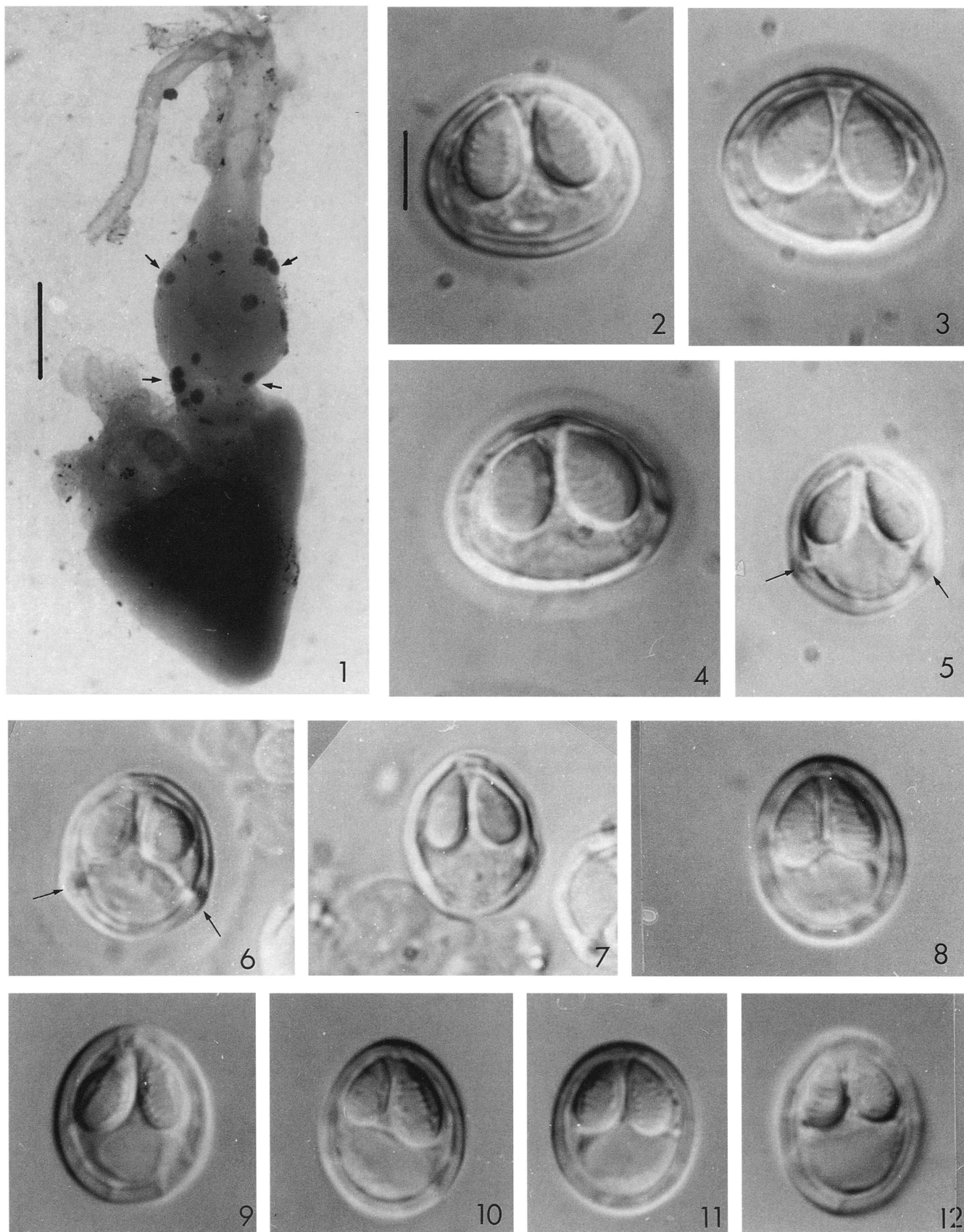
Etymology: This species is named in honor of Blair Jollimore of Hubbards, Nova Scotia, for a decade of assistance collecting fishes for parasitological studies.

Remarks

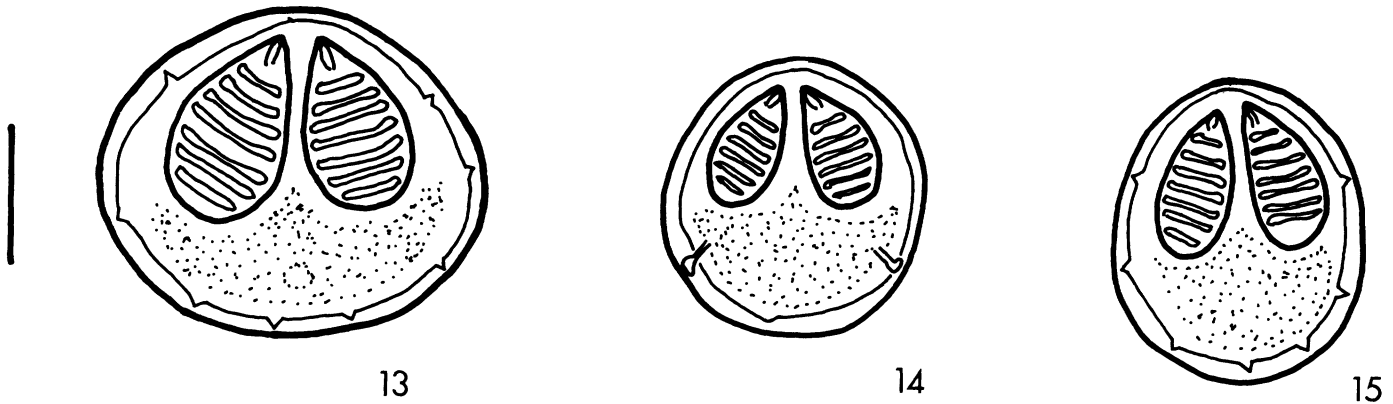
Spores of *M. jollimorei* are relatively large, subcircular spores with prominent polar capsules. They resemble spores of *Myxobolus filamentus* Grinham and Cone, 1990 (previously recognized as *Myxosoma okobojiensis* Rice and Jahn, 1943, which, when transferred to *Myxobolus*, became a homonym of *Myxobolus okobojiensis* Otto and Jahn, 1943 from *Pomoxis sparoides*) from gills of smallmouth buffalo (*Ictiobus bubalus*) and *Myxobolus magnasphaerus* Cone and Anderson, 1977 from the peritoneum of *L. gibbosus*. However, the spores of *M. filamentus* as originally described (see Fig. 3 of Rice and Jahn [1943]) are circular in frontal view as opposed to distinctly subcircular and have polar capsules that are longer relative to spore length (approximately 66% of spore length in *M. filamentus* versus 55–61% in *M. jollimorei*). Spores of *M.*

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FIGURES 1–12. Myxosporeans from the hearts of centrarchids. 1. Heart of *Myxobolus jollimorei* n. sp. from *Lepomis gibbosus* from the Pascagoula River system, Mississippi, with numerous pseudocysts (arrows) within tissues of the bulbus arteriosus. Scale bar 1 mm. 2–4. Developed spores of *Myxobolus jollimorei* n. sp. in frontal view. 5–7. Developed spores of *Myxobolus manuei* n. sp. from *Pomoxis nigromaculatus* in Lake



FIGURES 13–15. Line drawings depicting typical spores. Scale bar 5 μm . 13. *Myxobolus jollimorei* n. sp. 14. *Myxobolus manui* n. sp. 15. *Myxobolus paralintoni* Li and Desser, 1985.

magnasphaera have huge polar capsules relative to spore length (55–61% in *M. jollimorei* versus 58–68% for *M. magnasphaera*) (see fig. 3 of Cone and Anderson [1977]; plate 28K of Lom and Dyková [1992]).

***Myxobolus manui* n. sp.**

(Figs. 5–7, 14)

Description: Pseudocyst gray, spherical to saucer-shaped, 100–800 in diameter. Plasmodium lacking typical ectoplasm from periphery, with spores arranged loosely within interior. Spores subcircular in frontal view, $10.8.0 \pm 0.5$ (10–11) long, 9.12 ± 0.9 (8–10) wide, 7.0 ± 0.5 (6.5–7.0) thick. Spore width-to-length ratio $1:1.2 \pm 0.1$. Polar capsules pyriform, 5.3 ± 0.5 (4.5–6.0) long and 2.9 ± 0.8 (2.5–3.0) thick. Filament coils 6–7, wound tightly and perpendicular to longitudinal axis of capsule. Intercapsular appendix absent. Sutural ridge folds indistinct, with 2, short sublateral knobs along sutural ridge. Iodinophilous vacuole and mucous envelope lacking.

Taxonomic summary

Type host: Black crappie, *P. nigromaculatus* (Lesueur, 1829) (Centrarchidae).

Site of infection: Bulbus arteriosus, within the epipericardium and underlying smooth muscle and elastin. Free spores in kidney tissue and in the lumen of gall bladder.

Type locality: Lake Erie, near Wheatley, Ontario.

Type specimens: Syntypes are spores in Giemsa-stained sections in U.S. National Parasite Collection, Beltsville, Maryland, USNPC no. 87589.

Prevalence and intensity of infection: 57% (4 of 7) of black crappie (9.5 cm in total length and 21.3–22.3 cm in total length) collected from Lake Erie; each fish with 1–25 pseudocysts. The two black crappie (25.2 and 29 cm in total length) examined from the Pascagoula River system were not infected.

Etymology: This species is named in honor of Mr. Frank Manuel of Black Point, Nova Scotia, for his kind assistance to students conducting research under field conditions.

Remarks

The spore of *M. manui* n. sp. is unique among known species of *Myxobolus* from North America in having sublateral knobs along the sutural ridge. A similar sublateral “crest” was reported (Lom and Dyková, 1994) for *Myxobolus cone* Lom and Dyková, 1994 from the liver and bile ducts of *Pseudocaranx dentex* caught along the coast of New South Wales, Australia. However, *M. cone* differs significantly from *M. manui* in having coils (4 and sometimes 5 rather than 6–7) of the

polar capsules arranged $45\text{--}70^\circ$ to the longitudinal axis of the capsule rather than 90° .

***Myxobolus paralintoni* Li and Desser, 1985**

(Figs. 8–12, 15)

Supplementary data: Pseudocysts gray, spherical to saucer-shaped, 50–600 in diameter. Plasmodium with poorly defined outer ectoplasm; endoplasm containing loose mass of spores. Fresh spores subcircular in frontal view, 11.8 ± 0.8 (11.0–13.0, $n = 8$) long, 9.6 ± 0.4 (9.0–10.0) wide, and 5.0 thick. Spore width to length ratio $1:1.2 \pm 0.1$. Sutural ridge folds 6–8 in number, distributed evenly around the spore margin. Polar capsules pyriform, 5.2 ± 0.6 long and 2.9 ± 0.3 wide; filament coils 6–7 in number and perpendicular to the long axis of the capsule. Intercapsular appendix, mucus envelope, and iodophilous vacuole lacking in fixed specimens.

Taxonomic summary

Type host: Pumpkinseed, *L. gibbosus* (Linnaeus, 1758) (Centrarchidae).

Site of infection: Bulbus arteriosus, within the epipericardium and underlying tissue. Phagocytized spores in lumen of bulbous arteriosus.

Type locality: Lake Sasajewan, Algonquin Park, Ontario.

Examined specimens: Syntype slide, Canadian Museum of Nature, Parasite Collection, NMCICP 1984–0364; voucher specimens on hematoxylin and eosin-stained histological sections deposited in U.S. National Parasite Collection, Beltsville, Maryland, USNPC no. 87590.

Prevalence and intensity of infection: 30% (3 of 9) of *L. gibbosus* from Lake Sasajewan, and 57% (4 of 7) of this host from Inner Long Point Bay, Lake Erie, plus 1 of 7 specimens of *L. macrochirus* from Inner Long Point Bay. Infected fish had 1–38 pseudocysts.

Remarks

We examined syntypes of *M. paralintoni*. Although the slide with that material had dried and deteriorated, a spore was found and recognized as conspecific to those we collected from the type host (pumpkinseed) on the basis of spore size and shape; these additional specimens also came from the type locality of Lake Sasajewan. Spores in the original description (Li and Desser, 1985) were reported as 11 μm (9.5–11.5) long, 10.0 μm (9.0–11.5), and 6.5–7.5 μm thick, similar to those that we collected. Drawings of spores of *M. paralintoni* provided by Li and Desser (1985), however, resemble those of *Myxobolus uvu-liferis* Cone and Anderson, 1977 from the same host and lake. Rather, as described above, spores of *M. paralintoni* are subcircular in the plane of spore length. Recently, Hayden and Rogers (1997) reported *M. par-*

alintoni from the bulbus arteriosus of the redbreast sunfish (*Lepomis auritus*) in the Potomac River, Montgomery County, Maryland. Spores collected by Hayden and Rogers (1997) were identical in size and shape to material examined in the present study.

DISCUSSION

Myxobolus jollimorei, *M. manueli*, and *M. paralintoni* all have a plasmodium that develops specifically within tissues of the bulbus arteriosus of their respective hosts. Plasmodia were not found in any other organ. Histological sections of the heart revealed that spores can disperse from the trophozoite into the lumen of the bulbous arteriosus, where they are presumably put into general blood circulation. The presence of free spores in other organs is the result of such dispersal, and these organs should not be interpreted as alternate sites of plasmodial development. In this regard, the tissue specificity for the three species of *Myxobolus* appears more restricted than that of *Myxobolus bulbocordis* Masoumian, Baska and Molnar, 1996, which was reported (Masoumian et al., 1996) from the serosa of the atrium cordis, of the bulbus, and of the larger gill arteries, as well as inside the wall of the bulbus of *Barbus sharpeyi* in Iran (Masoumian et al., 1996). The restricted tissue development of *M. paralintoni* resembles more closely that of *Henneguya sebasta* Moser and Love, 1975 and *Henneguya* spp. that develop in the bulbus arteriosus of rockfish (*Sebastes* spp.) in the Pacific Ocean (Moser and Love, 1975; Moser et al., 1976) and bluefish (*Pomatomus saltatrix*) in the Atlantic Ocean (Meyers et al., 1977). Moser and Love (1975) and Moser et al. (1976) reported that the development of *H. sebasta* was restricted to the bulbus, but that free spores tended to aggregate in the lumen of the atrial and ventricular chambers, with isolated spores occurring in the gall and urinary bladders.

Myxosporean infections of the bulbus arteriosus appear to be characterized by significant spore dispersal beyond the plasmodium. This dispersal may be a result of the need for the bulbus arteriosus to expand significantly during the normal cardiac cycle; for rainbow trout (*Oncorhynchus mykiss*), 25% of the cardiac stroke volume is taken up by expansion of the bulbous arteriosus (Priede, 1976). This expansion would produce significant compression and stretching of the plasmodium, forces that may facilitate both rupturing of the plasmodium and dispersal of spores through tissues. We note that the plasmodia of the three myxosporeans reported here are saucer-shaped and only loosely filled with spores. Both these features could serve to minimize detrimental effects of compression during early stages of spore development.

The presence of 3 distinct species of *Myxobolus* in the bulbus arteriosus of 3 sympatric centrarchid fishes raises questions about possible historical relationships. Are these parasites the result of diversification of a species of *Myxobolus* that parasitized the bulbus arteriosus of an ancestral centrarchid? Or, are the three relatively distant phylogenetically, representing 3 independent invasions of a unique site within centrarchid fishes. The apparent rareness of species of *Myxobolus* parasitizing this tissue site in other fishes suggests the former hypothesis may be the case. However, the fact that each of the three species is different from each other morphologically and that each resem-

bles more closely species of *Myxobolus* from other hosts in different tissues than among themselves suggest the latter hypothesis may be the case. Molecular studies (e.g., Smothers et al., 1994; Siddall et al., 1995; Schlegel et al., 1996) on these 3 and additional species may help to answer this question.

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